Development of a new class of target cell lines to evaluate Fc-mediated Cytotoxicity

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INTRODUCTION

Early in the process of development of therapeutic antibodies, it is required to characterize their mode of action (MoA). In addition to the drugs specificity determined by the Fab moieties, their Fc regions can contribute to the drugs characteristics. Apart of playing a role in antibody half-life, the Fc region is determining the involvement of wanted or unwanted cytotoxic effects i.e., Antibody-Dependent Cell-mediated Cytotoxicity (ADCC), Antibody-Dependent Cellular Phagocytosis (ADCP) and Complement-Dependent Cytotoxicity (CDC). However, it is often difficult to evaluate which of these effects is prevalent in the mode of action.

In order to simplify this evaluation, we have established a target cell line, called CD20+SL allowing to evaluate ADCC, ACDP and CDC induction by a drug antibody. The CD20+SL cell line is based on a "Complement-Competent" cell line, we identified, engineered to constitutively express SVAR luciferase (SL).

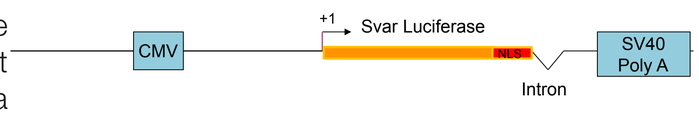
This cell line allows the development of fast, robust, and reproducible functional assays to assess CDC (by incubating with complement source), ADCC or ADCP when used in combination with ADCC and ADCP effector reporter cells based on luciferase detection. This target cell line can be easily customized to express any cell surface antigen of interest.

DESCRIPTION OF THE TARGET CELL LINE

The Complement-competent CD20+SL target cell line is stably and constitutively expressing the SVAR Luciferase reporter gene (SL) that has a nuclear localization signal (NLS) keeping the SL in the nucleus.

Only upon desintegration of the cells the Luciferase is released into the medium. Only in the medium the SL gets in contact with its substrate leading to cell-death-dependent light emission that can be detected by a luminometer.

Fig. 1
Schematic representation of the construction of the plasmid pLV-SNLS reporter gene introduced in the target cell line



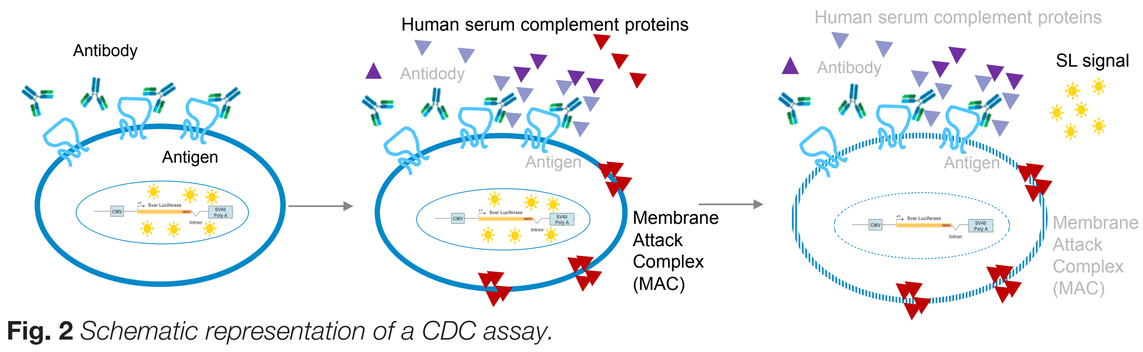
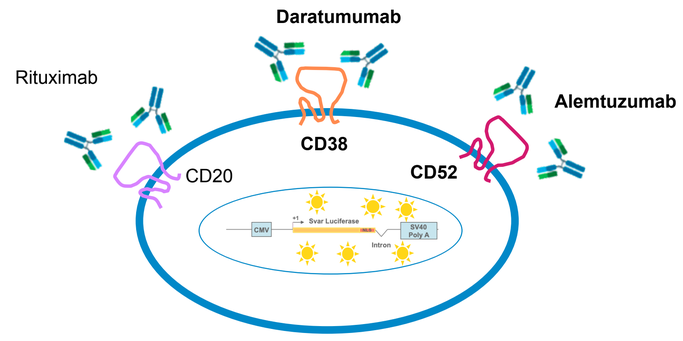


Fig. 2 *Schematic representation of a CDC assay.*

CDC ASSAY USING CD20+SL TARGET CELLS

The CD20+SL target cell line is endogenously expressing several relevant surface antigens recognized by drug antibodies capable of inducing complement-dependent cytotoxicity (CDC) such as CD20, CD38 and to a lesser extent CD52.



Here, we show that CDC-mediated killing can be readily detected using this target cell line.

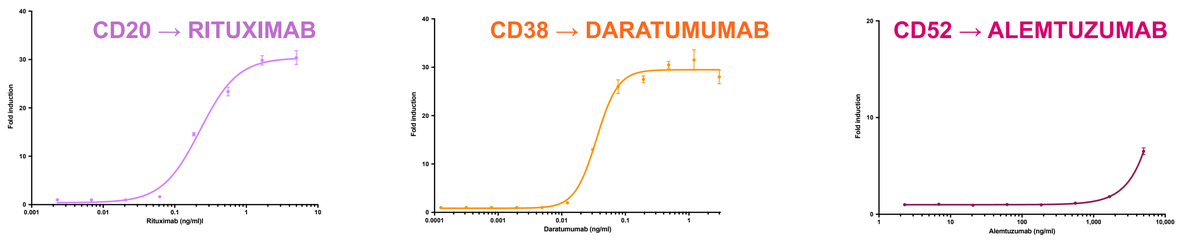


Fig. 3 *CDC induction by RITUXIMAB/ DARATUMUMAB/ALEMTUZUMAB*

ADAPTATION OF THE CD20+SL TARGET CELL LINE TO NON-ENDOGENEOUS SURFACE MARKERS

New target cell lines can be easily generated by introducing surface antigens into the CD20+SL target cell line.

This exercise has been made by introducing either membrane antigens such as ERBB2, EGFR or membrane bound versions of cytokines as mTNF or mVEGF.

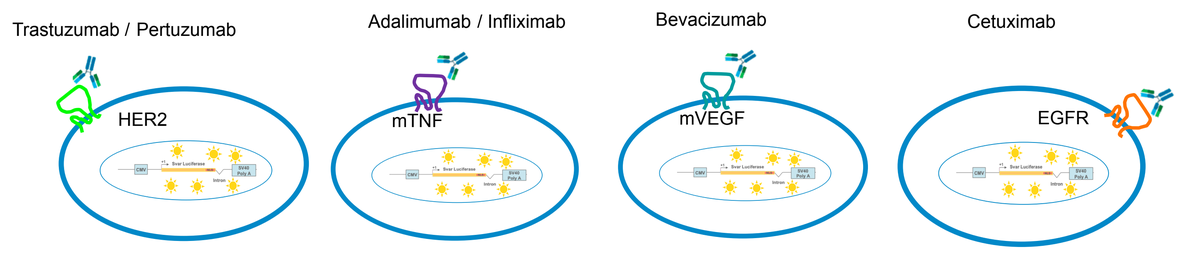


Fig. 4 *Schematic representation of the 4 new antigens introduced in the target cell line CD20+SL*

ADCC, ADCP & CDC ASSAY PERFORMED WITH THE SAME TARGET CELLS

ADCC, ADCP or CDC assays were performed using *iLite*® ADCC and ADCP effector cell lines or human serum, respectively.

The *iLite* effector cell lines are reporter cell lines expressing Firefly Luciferase as a reaction on antibody mediated and target-cell dependent cell signalling. Corresponding therapeutic antibodies have been tested to assess their degrees of involvement in each Fc-mediated mode of action.

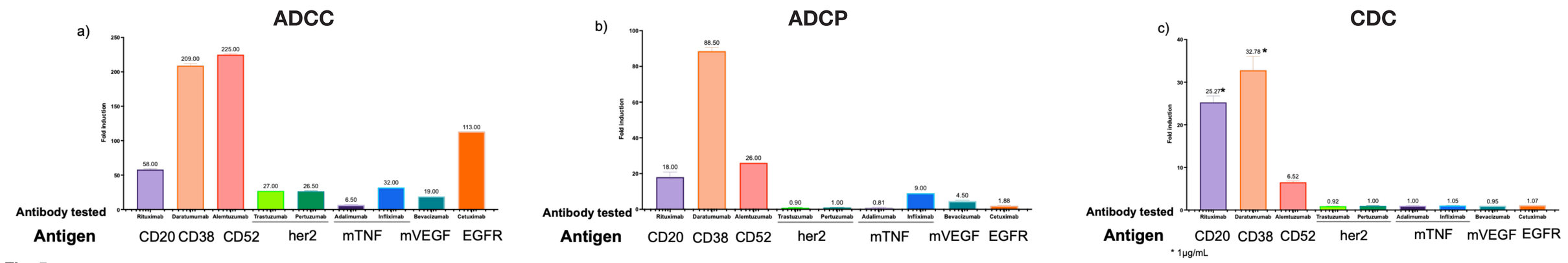


Fig. 5
Antibody-mediated ADCC and ADCP are measured by the activity of firefly Luciferase expressed by the iLite ADCC and ADCP effector cells, respectively. Antibody mediated CDC (c) is measured by the release of SVAR luciferase of the lysed cells. Of note, also in the context of ADCC and ADCP assays our CD20+SL target cells can be used in real killing assays. Data not shown here,

EFFECT OF ANTIBODY COMBINATIONS ON CDC INDUCTION

Different combinations of therapeutic antibodies have been tested for each antigen to evaluate a potential additive or synergistic effect to induce CDC. Here, we present results for CDC induction by 2 antibodies directed against Her2 (pertuzumab and trastuzumab) alone or in combination.

We repeatedly observe a weak but specific CDC induction on Her2 target cells when cells are incubated with both drug antibodies.

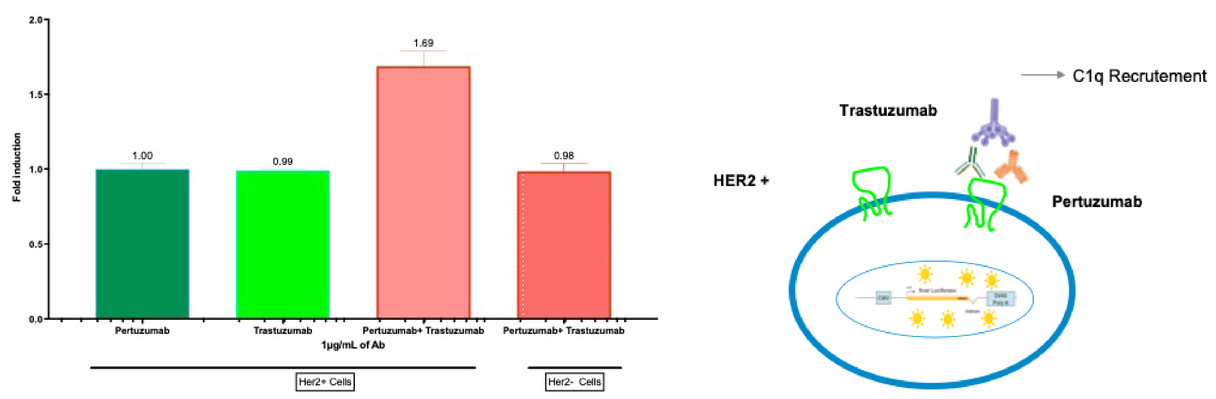


Fig. 6
CDC induction by different antibodies and combination of antibodies measured by the release of SVAR Luciferase by the target cell lines Her2+ and Her2-.

CONCLUSIONS/PERSPECTIVES

We established a target cell line allowing the development of fast, robust, and reproducible functional assays to detect Fc-mediated ADCC, ADCP and CDC based on luciferase detection.

The same unique/single target cell line is suitable for use in determining an antibodies CDC activity and assessing ADCC and ADCP activity in surrogate and killing assay setups.

The CD20+SL target cell platform allows fast customization of the target cells by adding surface antigens of interest thereby enabling the determination of Fc-mediated ADCC, ADCP and CDC of any antibody.

The CD20+SL is well suited for ADCC killing assays using PBMC-derived effector cells (data not shown here).

This versatile system is well-suited to detect (unwanted) Fc-mediated cytotoxic MoAs of therapeutic antibodies with agonistic, antagonistic or neutralising functions in functional assays using only one target cell line.

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